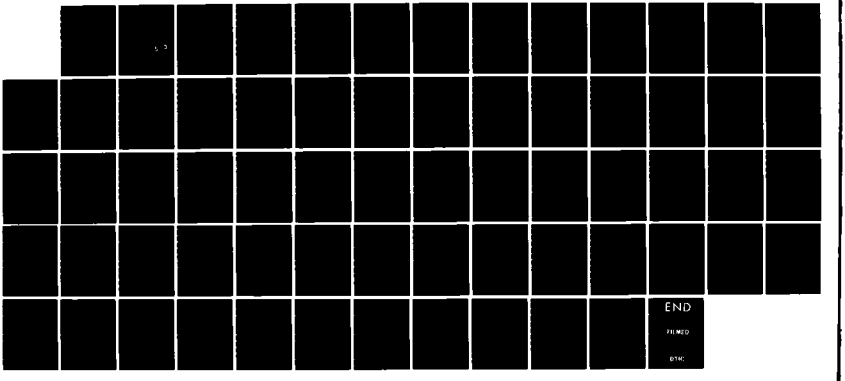
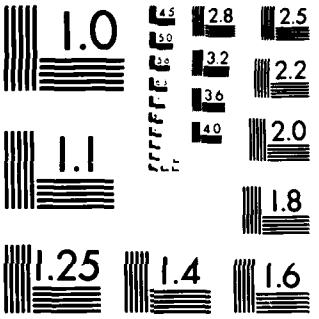
AD-A149 307 THE SCREENING AND EVALUATION OF EXPERIMENTAL ANTIPARASITIC DRUGS(U) MIAMI UNIV FLA LEO RANE RESEARCH LAB A AGER JUN 82 DAMD17-82-C-2022 1/1

UNCLASSIFIED F/G 6/15 NL





(12)

AD_____

REPORT NUMBER FOURTEEN

THE SCREENING AND EVALUATION OF EXPERIMENTAL ANTIPARASITIC DRUGS

Annual Report

Arba Ager, Ph.D.

June 1982

Supported by

US Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-82-C-2022

University of Miami
5750 NW 32nd Avenue
Rane Research Laboratory
Miami, Florida 33142

DOD Distribution Statement

Approved for public release; distribution unlimited.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: Fourteen	2. GOVT ACCESSION NO.: AD-A149307
3. RECIPIENT'S CATALOG NUMBER:	
4. TITLE *(and Subtitle)*: THE SCREENING AND EVALUATION OF EXPERIMENTAL ANTIPARASITIC DRUGS	5. TYPE OF REPORT & PERIOD COVERED: Annual Report 1 Oct 81 - 5 May 82
	6. PERFORMING ORG. REPORT NUMBER:
7. AUTHOR(s): Arba Ager, Ph.D.	8. CONTRACT OR GRANT NUMBER(s): DAMD17-82-C-2022
9. PERFORMING ORGANIZATION NAME AND ADDRESS: University of Miami, 5750 NW 32nd Avenue, Rane Research Laboratory, Miami, Florida 33142	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 62770A.3M162770A871.AF.041
11. CONTROLLING OFFICE NAME AND ADDRESS: US Army Medical Research and Development Command, Fort Detrick, Frederick, Maryland 21701	12. REPORT DATE: June 1982
	13. NUMBER OF PAGES: 57
14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)*:	15. SECURITY CLASS. *(of this report)*: Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE:

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Malaria	Exoerythrocytic	Trypanosoma rhodesiense
Plasmodium berghei	Prophylactic	African trypanosomiasis
P. yoelii	Repository	American trypanosomiasis
Drug-sensitive	Suppressive	Trypanosoma cruzi
Drug-sensitive	Mouse	Anopheles stephensi

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

See reverse side.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

ABSTRACT

The investigation undertaken during this report period included three antimalarial screens, two African trypanosome screens, and an American trypanosome screen. Three antimalarial screens were; a primary blood schizonticidal test using Plasmodium berghei, a primary causal prophylactic test using Plasmodium yoelii and Anopheles stephensi mosquitoes, and a secondary drug screening system using both of the above species of rodent malaria. The two African trypanosome systems included a primary screen and a secondary drug-resistant screen. The American trypanosome system was a primary one.

In the primary antimalarial blood schizonticidal test 1, 425 dose-level tests were done with 320 exhibiting activity. In the prophylactic antimalarial test 756 three dose-level tests were performed to detect activity against sporozoites and/or exoerythrocytic stages. 80 compounds were active. In the regular secondary 8 compounds were tested against the drug-sensitive P-line of P. berghei, while 12 compounds were tested against the drug mefloquine resistant-line. Other special tests included, 1) Determining how stable the drug-resistant lines would be when drug pressure is removed, 2) How stable drug resistance is when drug-resistant parasites are mixed with drug-sensitive parasites, 3) The development of mefloquine and Fansidar resistance when mixed together, 4) The activity of silver sulfonamide and its non-silver analog against malaria and African and American trypanosomes, 5) Testing a new prophylactic antimalarial test system using exoerythrocytic schizonts transfered from rat liver to mouse peritoneal activity.

The primary test in African trypanosomiasis evaluated compounds for trypanosomicidal activity against a drug-sensitive line. 935 three-level tests were performed with 17 compounds exhibiting activity. In the drug-resistant screen 30 compounds were tested for activity against one or more resistant lines. A special test was done to see how long resistance to melarsoprol would last when drug pressure was removed.

In the primary test in American trypanosomiasis 138 three-level tests were performed. Eight compounds were found to be active.

TABLE OF CONTENTS

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

ABSTRACT

The investigation undertaken during this report period included three antimalarial screens, two African trypanosome screens, and an American trypanosome screen. Three antimalarial screens were; a primary blood schizonticidal test using Plasmodium berghei, a primary causal prophylactic test using Plasmodium yoelii and Anopheles stephensi mosquitoes, and a secondary drug screening system using both of the above species of rodent malaria. The two African trypanosome systems included a primary screen and a secondary drug-resistant screen. The American trypanosome system was a primary one.

In the primary antimalarial blood schizonticidal test 1, 425 dose-level tests were done with 320 exhibiting activity. In the prophylactic antimalarial test 756 three dose-level tests were performed to detect activity against sporozoites and/or exoerythrocytic stages. 80 compounds were active. In the regular secondary 8 compounds were tested against the drug-sensitive P-line of P. berghei, while 12 compounds were tested against the drug mefloquine resistant-line. Other special tests included, 1) Determining how stable the drug-resistant lines would be when drug pressure is removed, 2) How stable drug resistance is when drug-resistant parasites are mixed with drug-sensitive parasites, 3) The development of mefloquine and Fansidar resistance when mixed together, 4) The activity of silver sulfonamide and its non-silver analog against malaria and African and American trypanosomes, 5) Testing a new prophylactic antimalarial test system using exoerythrocytic schizonts transfered from rat liver to mouse peritoneal activity.

The primary test in African trypanosomiasis evaluated compounds for trypanosomicidal activity against a drug-sensitive line. 935 three-level tests were performed with 17 compounds exhibiting activity. In the drug-resistant screen 30 compounds were tested for activity against one or more resistant lines. A special test was done to see how long resistance to melarsoprol would last when drug pressure was removed.

In the primary test in American trypanosomiasis 138 three-level tests were performed. Eight compounds were found to be active.

A SCREENING PROCEDURE FOR ASSESSING THE BLOOD SCHIZONTICIDAL ANTIMALARIAL ACTIVITY OF CANDIDATE COMPOUNDS IN PLASMODIUM BERGHEI INFECTED MICE

The recognition of chloroquine-resistant strains of Plasmodium falciparum in South America and Southeast Asia first posed what is now a critical problem in the chemotherapy of malaria. Parasite resistance to 4-aminoquinolines (e.g. chloroquine and amodiaquine), antifolates (e.g., pyrimethamine) and other standard antimalarial compounds such as quinine, has caused an increased concern for the development of safe alternative therapeutic agents.

The World Health Organization currently estimates that over 100 million cases of malaria worldwide require treatment each year. Recently, chloroquine-resistant parasites have been noted in Africa where over one million children die from malaria yearly. Reports from endemic areas in the world indicate a significant resurgence of malaria. The number of cases of malaria reported from Haiti exceeded 100,000 during 1981. During 1981 there were over 1,300 cases of malaria brought into the United States. The current widespread endemicity of malaria, with Chile the only country in South America and Central America free from malaria, and its potential for recurrence in malaria-free zones, the emergence of populations of parasites in Central and South America, Asia and Africa that are resistant to the major available antimalarial agents, and a decrease in vector control programs emphasize the need for continued mass screening of candidate antimalarial compounds.

A total of 284,560 three-level tests were performed from December 1, 1961 through April 30, 1982.

Table I summarizes the compounds tested and the mice used from December 1, 1961 through April 30, 1982.

The test system designed specifically for this operation is based on blood-induced Plasmodium berghei malaria infections in mice. It is a relatively simple and fast procedure. Assessment of antimalarial effect and host toxicity are reproducible and reliable.

All compounds evaluated were obtained from the Department of Parasitology at the Walter Reed Institute of Research and included: 1) Compounds structurally related to chemicals of known value as antimalarial agents; 2) Compounds structurally unrelated to compounds know to have antimalarial activity; 3) Structural analogues of compounds found active in our test system and representing several novel chemical groups; 4) Compounds known to have activity against other infectious disease agents.

Our own breeding colony of ICR/HA Swiss mice has continued to supply the animals used in our tests.

Drug activity was assessed by comparing the maximum survival time of treated malaria-infected animals to the survival time of untreated malaria-infected controls.

Using five and six week old mice and a standard inoculum of P. berghei, it has been possible to produce a consistently uniform disease fatal to 100% of untreated animals within six to seven days.

Since an established disease is less responsive to treatment than a disease in the early stages of development, treatment is withheld deliberately until a fairly high degree of parasitemia is evident. Test compounds are administered subcutaneously in a single dose on the third day post-infection at which time a 10-15% parasitemia has developed. A similar procedure is followed for the oral administration of selected active compounds.

To be classified as active, a compound must suppress the disease and produce an unquestionably significant increase, 100% or more, in the life span of the treated animals over that of the untreated controls. To be considered curative, treated animals must remain alive for 60 days after infection with P. berghei.

The severity of the challenges set up in our test system enhances the reliability of our evaluation and the antimalarial potential of the compounds selected to intensive preclinical studies.

METHODS

ANIMAL HOST

The total supply of animals needed to screen candidate compounds has been obtained from our breeding colony of ICR/HA Swiss mice (Mus musculus). Test animals weigh from 18-20 grams; weight variations in any given experimental or control group are carefully limited to within two to three grams. In any given test all animals are approximately the same age.

Animals on test are housed in metal-topped plastic cages, fed a standard laboratory diet and given water ad lib. Once the infected mice are given the drug, they are placed in a room maintained at 84°F (±2°F), and a relative humidity of 66% (±2%).

TEST PROCEDURE

Test animals receive an intraperitoneal injection of approximately 5.98×10^5 parasitized erythrocytes drawn from donor mice infected four days earlier with P. berghei. The donor strain is maintained by passing every four days in separate groups of mice inoculated with 0.2 cc of a 1:435 dilution of heparinized heart blood.

To check factors such as changes in the infectivity of our P. berghei strain or in the susceptibility of the host, one group of mice which serves as the negative control is infected but not treated. In order to determine the effect a drug exerts on a malaria infection, two parameters are measured; the first is an increase in survival time; the second concerns curative action. For comparative purposes, one standard compound, pyrimethamine, is administered at one level (120 mg/kg) to a group of 10 mice. Pyrimethamine serves as a positive control, producing definite increases in survival time and curative effects. Another function of the positive control involves monitoring three procedures: The drug weighing, the preparation of drug solutions and suspensions, and the administration of drugs.

DRUG ADMINISTRATION

Test compounds are dissolved or suspended in peanut oil before they are administered subcutaneously. Compounds to be administered orally are mixed in an aqueous solution of 0.5% hydroxyethylcellulose-0.1% Tween-80.

Treatment consists of a single dose given subcutaneously or orally three days post-infection. At the time of treatment, a 10-15% parasitemia has developed. Although the disease is well established, it has not yet caused sufficient debility to affect an evaluation of the test compound's toxicity.

Deaths that occur before the 6th day, when untreated controls begin to die, are regarded as the result of a compound's toxic effects and not as the result of action by the infecting parasite.

Each compound is initially administered in three graded doses diluted four-fold to groups of five mice per dose level. The top dose is 640, 320, or 160 mg/kg, depending on the amount of compound available for testing. Active compounds are subsequently tested at six or nine dose levels, diluted two-fold from the highest dose. Successive six-level tests are performed at respectively lower doses if necessary until the lower limit of activity is reached.

A drug that is toxic for the host at each of the three levels initially tested is retested at six dose levels diluted two-fold from the lowest toxic dose.

Increases in the dose levels of highly active compounds usually are followed by increases in the survival time of the treated mice. Treated animals alive at the end of 60 days are considered cured.

DRUG ACTIVITY

Acceptance of a drug as being sufficiently active for detailed studies is predicated on the margin between the maximum tolerated dose (MTD) and the minimum effective dose (MED) producing a significant effect. An MTD is defined as the highest

dose up to 640 mg/kg causing no more than one of five animals to die from drug toxicity. The MED is defined as the minimum dose increasing the life span of treated animals by 100% over the life span of untreated controls.

Clearly inactive compounds are rejected after one test, borderline compounds after two tests are rejected. Active compounds are characterized by a dose-response curve, which establishes the spread between the MTD and the lower limit of activity by a determination of drug activity in the dose level dilution tests. The total number of active compounds from December 1, 1961 to April 30, 1982 is summarized in Table II.

SPECIAL TEST WITH WR 228,258

This experiment was performed to explain previous obtained odd dose-response curves with WR 228,258 (BJ-07413). Mice were inoculated with P. berghei on D+0 and and the compound was administered once subcutaneously in peanut oil on D+3. Blood films were taken on days, 3, 5, 7, 9, 11, 13, 15, 17, 24, 31, 38, 45, 52, 59 and parasitemias determined. Toxicity control groups for each drug level received drug on D+3 but no parasites. Blood films were made from these mice the same time as those infected groups as a control for handling and cutting the tails of mice. An eratic dose-response curve was once again found. The number of mice surviving 60 days in both the infected and the non infected groups (toxicity controls) are tabulated below. There were two deaths at the lower doses in the toxicity controls. Whether these represent true drug toxicity deaths or deaths due to fighting is unknown. We are repeating this experiment using drug doses of 5, 2.5, 1.25 mg/kg plus toxicity controls in order to reach an endpoint in drug activity and verify any drug toxicity.

Mg/Kg WR 228,258	Number Mice Surviving 60 Days	
	Infected Group	Non Infected Group (Day died post treatment)
1280	2/10	10/10
640	1/10	10/10
320	0/10	10/10
160	0/10	10/10
80	1/10	10/10
40	4/10	10/10
20	6/10	10/10
10	5/10	9/10 (1 on D+5)
5	3/10	9/10 (1 on D+20)
- Control	0/10	10/10

PLASMODIUM BERGHEI MALARIA IN MICE

TABLE I

Summary of Screening Levels

December 1, 1961 - April 30, 1982

Time Period	Number of Three-Level Tests	Number of Mice
October 1, 1981 - April 30, 1982	1,415	21,700
October 1, 1980 - September 30, 1981	2,998	45,967
October 1, 1979 - September 30, 1980	4,826	74,040
October 1, 1978 - September 30, 1979	6,175	86,415
October 1, 1977 - September 30, 1978	5,375	82,690
June, 1976 - September, 1977	7,114	123,085
June, 1975 - May, 1976	9,916	155,585
June, 1974 - May, 1975	10,604	168,725
June, 1973 - May, 1974	11,035	168,664
June, 1972 - May, 1973	14,276	231,450
June, 1971 - May, 1972	14,874	262,245
June, 1970 - May, 1971	18,108	322,140
June, 1969 - May, 1970	22,376	411,270
June, 1968 - May, 1969	38,150	603,225
June, 1967 - May, 1968	40,465	636,525
June, 1966 - May, 1967	34,093	531,200
June, 1965 - May, 1966	22,731	350,449
June, 1964 - May, 1965	13,114	215,715
December, 1961 - May, 1964	6,915	250,000
TOTAL	284,560	4,741,090

PLASMODIUM BERGHEI MALARIA IN MICE

TABLE II

Summary of Active Compounds

June 1, 1970 - April 30, 1982

Time Period	Number of Three-Level Tests	Number of Active Tests
October 1, 1981 - April 30, 1982	1,415	320
October 1, 1980 - September 30, 1981	2,998	359
October 1, 1979 - September 30, 1980	4,826	581
October 1, 1978 - September 30, 1979	6,175	969
October 1, 1977 - September 30, 1978	5,375	1,261
June 1, 1976 - September 30, 1977	7,114	1,124
June 1, 1975 - May 31, 1976	9,916	351
June 1, 1974 - May 31, 1975	10,604	616
June 1, 1973 - May 31, 1974	11,035	394
June 1, 1972 - May 31, 1973	14,276	771
June 1, 1971 - May 31, 1972	14,874	593
June 1, 1970 - May 31, 1971	18,108	805
TOTAL	106,716	8,144

SECONDARY ANTIMALARIAL SCREENING SYSTEMS

Current prospects for the control of human malaria have been complicated by the occurrence of drug-resistant parasites. Such resistance falls into three categories, namely:

1. Resistance to antifolic drugs (pyrimethamine, chloroguanine, etc.).
2. Resistance to 4-aminoquinolines and acridines (chloroquine, amodiaquine, atebrine, quinine, etc.).
3. A combination of 1 and 2 which is referred to as multiple resistance.

Collectively, the several types of resistance impair the effectiveness of all major suppressive drugs. Hence, a great need exists for alternative drugs as well as new combinations of drugs.

New candidate compounds are emerging from a primary blood schizonticidal screening program, and it is particularly important to determine quite early which of the new candidates are likely to be useful against the various types of drug-resistant malaria. Experience has indicated that plasmodia of animals can be used for this purpose.

The specific aims for this test system were to conduct a sequential battery of chemotherapeutic studies in Plasmodium berghei infected mice on active compounds (discreet or open) emerging from the Department of Defense sponsored screening programs in order to determine which substances were worthy of further consideration as potential agents for dealing with drug-resistant malaria.

METHODS

The techniques used in this secondary drug testing program fell into two categories which are:

1. Studies designed to determine if a new agent was likely to be useful against the various types of drug-resistant malaria.

2. General chemotherapeutic characterization of selected new agents to suggest optimal methods of use and specific purposes they may serve.

The testing was done with P. berghei in outbred ICR/HA female Swiss mice (Mus musculus) weighing 20-25 grams. Briefly, this testing entailed procedures for the direct assessment of the effects of drugs on the parasitemia. Various gross tolerance observations were also recorded which served as guides indicating the usefulness of the new test agents as drugs for the treatment of malaria.

More specifically, activities included elucidation of the apparent mode of action of agents by testing them in parallel against drug-sensitive P. berghei (KBG-173) and various drug-resistant derivatives of this malaria strain. The six drug-resistant derivatives included a chloroquine-resistant, a cycloguanil-resistant, a dapsone-resistant, a mefloquine-resistant, a pyrimethamine-resistant and quinine-resistant line.

TEST DESIGN

When a new compound is obtained it is subjected to a battery of testing procedures, the extent of which depends on its degree of activity in suppressing murine malaria infections. The first test procedure is a 6-day suppressive test against the drug-sensitive P-line.

If the compound is active against the P-line, then a 6-day test against one or more drug-resistant lines follows. In this basic 6-day suppressive test, mice are divided into groups of seven and inoculated with parasites intraperitoneally. Drugs are administered twice a day, usually orally, in a volume of 10 ml/kg on the third, fourth and fifth days after inoculation of parasites. All drugs are mixed in aqueous 0.5% hydroxyethylcellulose-0.1% Tween-80 and ultrasonicated when neccesary. Drug doses are prepared using 100% of the free base of each drug. One group of ten infected mice receives the vehicle alone and serves as a negative control. The blood films and

final group weights are taken on the sixth day after inoculation of parasites. Microscope examination of Giemsa-stained blood smears is made to determine the percentage of cells parasitized, percent suppression of parasitemias, and significance values for the suppression of parasitemias. Significance values are based on a calculation of the percent suppression of parasitemia which is determined by comparing the parasitemia of each treated mouse with the mean parasitemia of the negative controls. Drugs tolerance is reflected by the percent weight change and the proportion of mice that survive treatment. Toxicity is attributed to drug action when a - 14% or greater weight change occurs or when one or more mice die before the blood smears are taken.

REGULAR P-LINE TESTING

Each new drug is tested first against the drug-sensitive P-line usually via both oral and subcutaneous routes of administration. The drug dosages for the first test are normally 64, 16, 4 and 1 mg/kg/day for three days. If more than a 90% suppression of the parasitemia (SD_{90}) is obtained with the lower dose of 1 mg/kg/day, then testing at lower doses is performed. Chloroquine is tested as a reference against the P-line at levels of 2, 3, and 4 mg/kg/day. A quinine index (Q) is calculated by comparing the SD_{90} value obtained from the chloroquine dose-response curve and the SD_{90} value of the new compound:

$$Q = \frac{SD_{90} \text{ of chloroquine}}{SD_{90} \text{ of new compound}} \times 30^{*}$$

* = This is the quinex index for chloroquine

DRUG-RESISTANT LINES

Compounds that suppress the P-line parasitemia by at least 90% with 64 mg/kg or less are subjected to testing against one or more of the six drug-resistant lines. These lines include a chloroquine-resistant (C-line), a cycloguanil-resistant (T-line), a dapsone-resistant (S-line), a mefloquine-resistant (A-line), pyrimethamine-resistant (M-line) and a quinine-resistant line (Q-line). The amount of testing against the resistant lines depends upon the structure of each new compound as it relates to the

structure of known antimalarials. A maximum dose of 256 mg/kg/day is administered orally along with doses of 64, 16 and 4 mg/kg/day.

ESTIMATES OF POTENCY AND CROSS RESISTANCE

Doses required for a given degree of effect, such as 90% suppression or SD_{90}'s are estimated graphically from plots made on log-probit paper. The ratio of the SD_{90}'s are used to delinete the degree of cross resistance.

RESULTS

Twenty-one compounds have been tested in the secondary test system; 12 compounds vs. the A-line, and 8 compounds against the P-line. One compound (WR 1,504) was tested against both the P-line and A-line. A summary of compounds tested is presented in Table III.

SPECIAL TESTS

THE LOSS OF DRUG RESISTANCE OF 6 LINES OF P. BERGHEI ASSOCIATED WITH REMOVAL FROM DRUG PRESSURE

Rodent malaria (P. berghei KBG-173 strain) has the ability to develop resistance to a number of commonly used anti-malarial chemotherapeutic agents. The A-line is resistant to mefloquine (WR 142,490) C-line is resistant to chloroquine (WR 1,544), M-line is resistant to pyrimethamine (WR 2,978), Q-line is resistant to quinine (WR 2,976), S-line is resistant to dapsone (WR 448) and T-line is resistant to cycloguanil (WR 5,473).

This report is interested in the time it takes for each of these six drug-resistant lines to lose resistance to their respective drugs. The six malaria lines that were removed from drug pressure were tested, at intervals of 1, 2, 3, 6, 9, 10, 15 and 18 months for loss of resistance by comparison with the original drug-resistant line maintained under drug pressure and the drug-sensitive P-line.

METHODS

The drug-resistant lines were maintained by passing the parasites either once a week (M and T-lines) or every second week (A,C,Q, and S-lines) to mice that have free access to drug mixed in with laboratory chow. In the case of A-line, mice were gavaged three times a week with 0.28 mg/kg with mefloquine. The lines away from drug pressure were maintained by passing the parasites either once a week (PYR* and T* - lines) or every second week (A*, C*, Q* and S* - lines).

Female ICR/HA Swiss outbred mice weighing 20-25 g. were infected either with $15X10^6$ parasitized red blood cells (A, A*, C, C*, Q, Q*, S, S* - lines) on day 0 or 0.05×10^6 parasitized red blood cells (M, M*, T, T* and P-lines) on day 0.

Drug treatment began on day 4. A and A* - lines received mefloquine orally twice a day totaling 256, 64, 16, 4 or 1 mg/kg for three days. C and C* lines received chloroquine twice a day totaling 256, 64, 16, 4, 2 or 1 mg/kg for three days. M and M* - lines received pyrimethamine twice a day totaling 100, 25, 6.25, 1.56, 0.39 or 0.09 mg/kg for three days. Q and Q* received quinine orally twice a day totaling 256, 128, 64 or 32 mg/kg for three days. S and S* - lines received dapsone orally twice a day totaling 256, 64, 16, 4, 1 or 0.25 mg/kg for three days. T and T* - lines received cycloguanil orally twice a day totaling 256, 64, 16, 4 and 1 mg/kg for three days.

Giemsa-stained blood smears were obtained the day after the last drug treatment (day 7) and the percent parasitemia was determined for each mouse. Percent suppression of parasitemia and the SD_{90}'s, and SD_{70}'s were calculated from this data. The degree of resistance for each of the lines was calculated for each monthly interval.

RESULTS

Doses required for a given degree of effect, such as 90% and 70% suppression, or SD_{90} and SD_{70}, respectively, are estimated graphically from plots made on log-probit paper (Table IV). The ratio of the SD_{90}'s are used to delineate the degree

of cross resistance (Table V).

The C* - line and M* - line began to lose resistance to their respected drugs (chloroquine and pyrimethamine) during the period between 9 and 12 months after removal from drug pressure. The C* - line dropped from a SD_{90} of >256 mg/kg/day at 9 months to a SD_{90} of 2.7 mg/kg/day by 12 months. During the same time period the degree of resistance dropped from a value of >106.7 to 1.1. During the ensuing tests the degree of resistance has remained at 1.0. The M* - line dropped from a SD_{90} of 66 mg/kg/day at 9 months to a SD_{90} of 0.26 mg/kg/day by 12 months. During this period the degree of resistance dropped from a value of 227.6 to 1.0. It has since remained at 1.0.

The A* - line, S* - line, T* - line and Q* - line all remain resistant to their respective drugs 18 month after removal from drug pressure.

THE STABILITY OF RESISTANCE IN DRUG-RESISTANT LINES WHEN MIXED WITH DRUG-SENSITIVE PARASITES

The purpose of this study was to see if drug-sensitive malarial parasites would outcompete and overgrow drug-resistant parasites. Drug-sensitive parasites were mixed with drug-resistant parasites then inoculated together into mice. Tests to determine if drug resistance was lost were performed at 0, 1, 2 and 3 month.

MALARIA LINES

All the rodent malarial lines were developed from the KBG-173 line of Plasmodium berghei.

P + T-line = 0.05×10^6 P-line + 0.05×10^6 T-line
P + M-line = 0.05×10^6 P-line + 0.05×10^6 M-line
P + C-line = 0.05×10^6 P-line + 15×10^6 C-line
P + A-line = 0.05×10^6 P-line + 15×10^6 A-line

Each of the following lines were passed at weekly intervals P, T', M', P+T, P+M, P+C, P+A, T°, M°. Two lines were passed biweekly (C°, and A°)

TESTING FOR DRUG RESISTANCE

Four lines (P+T, P+M, P+C, and P+A) were started with similar mixtures of parasites. They were inoculated i.p. into mice (ICR/HA) for initial testing of drug resistance (0 month test). The lines were maintained for one month without drug pressure, a second test was performed to see if the drug resistance patterns had changed in the 4 mixture lines. For this testing and subsequent testing to determine degrees of cross resistance, an inoculum in mice of 0.05×10^6 parasitized red blood cells was used for each of the 4 mixture lines.

Each test to assess the degrees of resistance was a regular 6-day test for detection of suppressive activity. Mice were infected on D+0 and test compounds were administered b.i.d. on D+3, D+4 and D+5. Blood films were taken on D+6 and parasitemias determined. SD_{90} values were determined from plots on probit-log paper.

RESULTS

0 MONTH

The SD_{90} values obtained in the initial test showed each mixture of drug-resistant parasites with drug-sensitive parasites to be fully resistant to their respective drug (Table VI).The mixture of P+T was just as resistant to cycloguanil as the T'line. Similar results were obtained with the mixtures of P+M, P+C, and P+A.

1 MONTH

Each of the 4 drug-mixture lines (P+M, P+T, P+C, P+A) lost their respective drug resistance within one month (Table IV). The 4 drug-resistant lines (M°, T°, C° and A°) removed from drug pressure and passed for 1 month did retain their respective drug resistance. Some mefloquine-resistant parasites remained in the P+A line because 100% suppression was not attained.

2 AND 3 MONTHS

The drug-mixture lines remained stable and did not exhibit any resistance to their respective compounds(Table VI). The 4 drug-resistant lines removed from drug pressure remained fully resistant to their respective drugs. Some mefloquine-resistant parasites remained in the P+A line because 100% suppression was not attained.

SPECIAL EXPERIMENTS WITH MEFLOQUINE AND FANSIDAR AND PLASMODIUM YOELII

A series of lines were placed under drug pressure to verify Dr. Peters claim that the acquisition of resistance to mefloquine would be slowed down when administered in combination with Fansidar (a combination of pyrimethamine and sulfadoxine).

The test plan was to use three groups of mice in the following plan:

	Group 1	Group 2	Group 3
Mefloquine	4 mg/kg	-	2 mg/kg
Sulfadoxine	-	0.03125 mg/kg	0.03125 mg/kg
Pyrimethamine	-	0.0625 mg/kg	0.0625 mg/kg

The mice were initially infected with $1.0X10^6$ parasitized red blood cells of the drug-sensitive Plasmodium yoelii line. The test compounds were administered once a day on D+3, D+4, and D+5. Each pass was made from mice with a parasitemia in the range of 1 to 10%. Drug pressure was increased initially at each pass, however, this proved to be too frequent. The concentration of drugs administered and number of passes at each level is presented in Table VII.

The first test for resistance was performed at the 20th pass (Exp. 318). Resistance to mefloquine (at the SD_{50} mg/kg level) was observed to be less in the

triple combination than when mefloquine was administered alone. The SD_{50} of mefloquine in the triple combination was 35 mg/kg while a value of >256 mg/kg was found in the line receiving only mefloquine. Another test for resistance was at the 24th pass (Exp. 321). Complete resistance to mefloquine at the 256 mg/kg level was observed in both the lines receiving mefloquine (the mefloquine only line and the triple combination line).

A second series of the three lines was started and a test for resistance was performed at the 8th pass (Exp. 321). A slight increase in resistance at the SD_{90} level (3-fold) was found in the line receiving only mefloquine. The triple combination line did not exhibit any resistance to mefloquine.

We have also started three additional sets of 3 test groups (9 boxes) with the hope of increasing mefloquine pressure at more frequent intervals.

TEST TO DETERMINE ACTIVITY OF A SILVER SULFONAMIDE (WR 261,560) AND ITS NON-SILVER ANALOG (WR 261,533).

Three special tests were performed with a silver sulfonamide (WR 261,560 BK-01907) and its non-silver anolog (WR 261,553 BK-01890) to examine if they had any antiparasitic activity against the following parasites;

Plasmodium berghei asexual erythrocytic stages (MM Test)

Plasmodium yoelii asexual exoerythrocytic schizonts and or sporozoites (RP Test)

Trypanosoma rhodesiense trypomastigotes stages (RR Test)

Trypanosoma cruzi trypomastigote and or amastigote stages (TC Test).

Each compound was administered orally at 400 mg/kg per dose daily (at 6 hour intervals). The drug administration began 6 hours after parasite inoculation and continued for 7 days or until the mice died, which ever came first. In the first experiment the compounds were suspended in 0.5%hydroxyethylcellulose-0.1% Tween (HEC/Tw) while in the last two experiments the compounds were suspended in water. Infected untreated control mice were given (HEC/Tw). Mortality figures were recorded daily. For each parasite 15 mice were treated. There was no antiparasitic activity

observed for either compound tested against each of the four different parasites. All the mice died within the same time period as the infected untreated controls.

TABLE III

Summary of Data of Compounds Tested in Secondary Test System

October 1, 1981 - April 9, 1982

WR No. Bottle No.	Exp. No.	SD_{90} (mg/kg/day)[1] P	SD_{90} (mg/kg/day)[1] A	Rx[2]	Z[3]	Q[4]
1,544	309	2.7		P.O.		
AR-20613	309	2.4		S.C.		
	311		>128			
2,978	309	<0.25		P.O.		
AG-65046	309	<0.25		S.C.		
142,490	310		>256	P.O.	>64.0	
BG-39453	311		>256	P.O.	>77.5	
169,626 BK-09350	310		45	P.O.	2.0	
214,403 BE-14776	311		>256	P.O.	>6.0	
230,190 BG-85373	311		14.3	P.O.	1.0	
231,533 BG-94952	310		10.5	P.O.	1.0	
232,704 BH-05692	310		11.5	P.O.	1.0	

TABLE III (Cont.)

WR No. Bottle No.	Exp. No.	SD_{90} (mg/kg/day)[1] P	SD_{90} (mg/kg/day)[1] A	Rx[2]	Z[3]	Q[4]
233,154 BH-09387	311		90.0	P.O.	3.2	
233,436 BH-12946	310		12.5	P.O.	1.0	
233,545 BH-13345	310		3.3	P.O.	0	
234,579 BH-27072	311		1.7	P.O.	0	
235,325 BH-35056	311		98.0	P.O.	2.9	
242,511	309	0.09		P.O.		1.0
BJ-78592	309	0.16		S.C.		
242,745	309	0.25		P.O.		1.0
BH-96237	309	0.48		S.C.		
247,170 BJ-86423	310		>256	P.O.	>1	
248,399	319	>2-Toxic		S.C.		
BK-02039	320	Toxic		S.C.		
248,400	319	>8		S.C.		
BK-02048	321	>64		S.C.		
	322	200		S.C.		

TABLE III (Cont.)

WR No. Bottle No.	Exp. No.	SD_{90} (mg/kg/day)[1] P	A	Rx[2]	Z[3]	Q[4]
250,050	323	7.6		S.C.		
BK-16793	323	9.5		P.O.		6.8
250,051	323	22.0		S.C.		
BK-16800	323	9.2		P.O.		7.0
250,052	323	24.5		S.C.		
BK-16784	323	20.0		P.O.		3.2

[1] Amount of drug to suppress 90% of the parasites for the following lines: P=drug-sensitive; A=mefloquine-resistant.

[2] P.O. = Oral. S.C. = Subcutaneous.

[3] Z = Degree of resistance = $\frac{SD_{90} \text{ drug-resistant line}}{SD_{90} \text{ drug-sensitive line}}$

[4] Q = Quinine Index = potency relative to quinine against sensitive parasites (P-line) via the oral route of administration.

TABLE IV

Comparison of suppressive activities of drug-resistant parasites away from drug pressure with drug-resistant parasites maintained on drug pressure.

	Months away from drug pressure											
	1			2			3			6		
	P[2]	A*[3]	A[4]	P	A*	A	P	A*	A	P	A*	A
SD_{90}[1]	4	>256	>256	4.1	>256	>256	11	>256	>256	2.5	>256	>256
	P[2]	C*[3]	C[4]	P	C*	C	P	C*	C	P	C*	C
SD_{90}	1.9	>256	>256	3.1	10	>256	3.3	>256	>256	2.9	>256	>256
	P[2]	M*[3]	M[4]	P	M*	M	P	M*	M	P	M*	M
SD_{90}	2.3	60	66	1.2	>100	76	1.3	40	75	0.19	99	98
	P[2]	Q*[3]	Q[4]	P	Q*	Q	P	Q*	Q	P	Q*	Q
SD_{90}	82	235	>256	100	210	115	150	235	>256	150	220	210
	P[2]	S*[3]	S[4]	P	S*	S	P	S*	S	P	S*	S
SD_{90}	2.6	100	250	0.8	>256	>256	2.4	256	200	1.5	>256	180
	P[2]	T*[3]	T[4]	P	T*	T	P	T*	T	P	T*	T
SD_{90}	21	>256	>256	9.2	>256	>256	27	>256	>256	27	>256	>256

TABLE IV (Cont.)

	Months away from drug pressure											
	9			12			15			18		
	P[2]	A*[3]	A[4]	P	A*	A	P	A*	A	P	A*	A
SD_{90}[1]	2.9	>256	>256	6.2	>256	>256	3.3	>256	>256	3.6	>256	>256
	P	C*	C	P	C*	C	P	C*	C	P	C*	C
SD_{90}	2.4	>256	>256	2.1	2.7	>256	2.4	1.75	>256	2.4	2.4	>256
	P	M*	M	P	M*	M	P	M*	M	P	M*	M
SD_{90}	0.29	66	76	0.70	0.36	>100	0.68	0.27	>100	0.23	0.22	>100
	P	Q*	Q	P	Q*	Q	P	Q*	Q	P	Q*	Q
SD_{90}	82	>256	>256	98	>256	>256	82	>256	>256	77.0	208	>256
	P	S*	S	P	S*	S	P	S*	S	P	S*	S
SD_{90}	1.1	>256	>256	2.2	>256	>256	1.3	>256	>256	0.47	>256	>256
	P	T*	T	P	T*	T	P	T*	T	P	T*	T
SD_{90}	11.0	>256	>256	14.0	>256	>256	20	>256	>256	1.0	155	>256

1 SD_{90} = Mg/Kg day of compound to attain 90% suppression of parasitemia.

2 P - Drug-sensitive line

3 A*, C*, M*, Q*, S*, T* - Drug-resistant malaria lines removed from drug pressure.

4 A, C, M, Q, S, T - Drug-resistant malaria lines maintained on drug pressure.

TABLE V

A comparison of degrees of resistance at SD_{90} of drug-resistant parasites away from drug pressure for 1, 2 and 3 months with drug-resistant parasites maintained on drug pressure

Months away from drug pressure					
1		2		3	
A*[1]	A[2]	A*	A	A*	A
>64	>64	>62.4	>62.4	>23.4	>23.4
C*[1]	C[2]	C*	C	C*	C
134.7	>134.7	3.0	>82.5	>75.8	>75.8
M*[1]	M[2]	M*	M	M*	M
26.1	28.7	>83.3	63.3	30.8	57.7
Q*[1]	Q[2]	Q*	Q	Q*	Q
2.9	>3.1	2.1	1.2	1.6	>1.7
S*[1]	S[2]	S*	S	S*	S
38.5	96.2	>320	>320	>106.6	83.3
T*[1]	T[2]	T*	T	T*	T
>12.2	>12.2	>27.8	>27.8	>9.5	>9.5

[1] A*, C*, M*, Q*, S*, T* - Drug-resistant malaria lines removed from drug pressure.

[2] A, C, M, Q, S, T - Drug-resistant malaria lines maintained on drug pressure.

TABLE V (Cont.)

A comparison of degrees of resistance at SD_{90} of drug-resistant parasites away from drug pressure for 6 and 9 months with drug-resistant parasites maintained on drug pressure

Months away from drug pressure			
6		9	
A*[1]	A[2]	A*	A
>102.4	>102.4	>88.3	>88.3
C*[1]	C[2]	C*	C
>88.3	>88.3	>106.7	>106.7
M*[1]	M[2]	M*	M
521.0	521.0	227.6	262.1
Q*[1]	Q[2]	Q*	Q
1.5	1.4	>3.1	>3.1
S*[1]	S[2]	S*	S
>170.7	120	>232.7	>232.7
T*[1]	T[2]	T*	T
>9.5	>9.5	>23.3	>23.3

TABLE V (Cont.)

A comparison of degrees of resistance at SD_{90} of drug-resistant parasites away from drug pressure for 12, 15 and 18 months with drug-resistant parasites maintained on drug pressure

Months away from drug pressure					
12		15		18	
A*	A	A*	A	A*	A
>41.3	>41.3	>77.6	>77.6	>71.1	>71.1
C*	C	C*	C	C*	C
1.1	>106.7	1.0	>121.9	1.0	>106.7
M*	M	M*	M	M*	M
1.0	>142.9	1.0	>147.1	1.0	>438.8
Q*	Q	Q*	Q	Q*	Q
>2.6	>2.6	>3.1	>3.1	2.7	>3.3
S*	S	S*	S	S*	S
116.3	116.3	>196.9	>196.9	>544.7	>544.7
T*	T	T*	T	T*	T
>18.3	>18.3	>128	>128	155	>256

TABLE VI

Loss of drug resistance when drug-sensitive parasites were mixed with drug-resistant parasites.

Malaria Lines	0 mo		1 mo		2 mo		3 mo	
	SD_{90}	°Resist	SD_{90}	°Resist	SD_{90}	°Resist	SD_{90}	°Resist
P[a]	6.6		5.4		5.4		5.5	
T'[b]	>256	>38.8	>256	>47.4	>256	>47.4	>256	>46.5
T°[f]			>256	>47.4	>256	>47.4	>256	>46.5
P+T[j]	>256	>38.8	3.4	< 1.0	6.0	1.1	8	1.5
P[a]	0.41		<0.39		0.45		0.5	
M'[c]	>100	>244	>100	>256	70.0	155.6	65.0	130.0
M°[g]			99	>253.8	>100.0	222.2	99.0	198.0
P+m[k]	>100	>244	<0.39	1.0	<0.39	<1.0	0.42	<1.0
P[a]	1.9		1.3		1.9		1.7	
C'[d]	>256	>134.7	>256	>196.9	>256	>134.7	>256	>150.6
C°[h]			>256	>134.7	>256	>134.7	40.0	23.5
P+C[l]	>256	>134.7	3.1	2.4	2.2	1.2	2.4	1.4
P[a]	2.8		3.0				2.6	
A'[e]	>256	>91.4	>256	>85.3			>256	>98.5
A°[i]			>256	>64.0	>256	>23.3	>256	>98.5
P+A[m]	>256	>91.4	3.5	1.2			3.8	1.5

[a] P-Drug-sensitive line

[b] T'-Cycloguanil-resistant line (SD_{90} of cycloguanil = >256mg/kg/day)

[c] M'-Pyrimethamine-resistant line (SD_{90} of pyrimethamine = >125mg/kg/day)

[d] C'-Chloroquine-resistant line (SD_{90} of chloroquine = >256mg/kg/day)

[e] A'-Mefloquine-resistant line (SD_{90} of mefloquine = >256mg/kg/day)

TABLE VI (Cont.)

[f] T°-Cycloguanil-resistant line without cycloguanil drug pressure

[g] M°-Pyrimethamine-resistant line without pyrimethamine drug pressure

[h] C°-Chloroquine-resistant line without chloroquine drug pressure

[i] A°-Mefloquine-resistant line without mefloquine drug pressure

[j] P+T mixture of drug-sensitive and cycloguanil-resistant parasites

[k] P+M mixture of drug-sensitive and pyrimethamine-resistant parasites

[l] P+C mixture of drug-sensitive and chloroquine-resistant parasites

[M] P+A mixture of drug-sensitive and mefloquine-resistant parasites

TABLE VII

Development of mefloquine and Fansidar resistance by Plasmodium yoelii with fixed concentration of mefloquine and Fansidar.

	Mg/Kg/Day Drug			
Drug	Box 1	Box 2	Box 3	Pass #
Mefloquine	4	-	2	1 - 3
Sulfadoxine	-	0.03125	0.03125	
Pyrimethamine	-	0.0625	0.0625	
Mefloquine	8	-	4	4 - 5
Sulfadoxine	-	0.0625	0.0625	
Pyrimethamine	-	0.125	0.125	
Mefloquine	16	-	8	6 - 14
Sulfadoxine	-	0.125	0.125	
Pyrimethamine	-	0.25	0.25	
Mefloquine	32	-	16	15 - 16
Sulfadoxine	-	0.25	0.25	
Pyrimethamine	-	0.5	0.5	
Mefloquine	64	-	32	17 - 21
Sulfadoxine	-	0.5	0.5	
Pyrimethamine	-	1.0	1.0	
Mefloquine	128	-	64	22 - 26
Sulfadoxine	-	1.0	1.0	
Pyrimethamine	-	2.0	2.0	

SPOROZOITE INDUCED ANTIMALARIAL TEST IN MICE

Primaquine is the only drug currently used today for radical curative activity in humans (elimination of existing exoerythrocytic schizonts of Plasmodium vivax and Plasmodium ovale). This 8-aminoquinoline has two major limitations; First is its poor therapeutic index, and second is concerning its involvement in causing hemolytic anemia in persons with a deficiency in the enzyme glucose-6-phosphate dehydrogenese. New active 8-aminoquinolines as well as other groups of chemicals exhibiting casual prophylatic and radical curative activity are needed to combat malaria in the world today.

This test is intended to serve as a primary screening procedure for compounds submitted by the Department of Parasitology, Division of Experimental Therapeutics at the Walter Reed Army Institute of Research.

In this test system mice receive a subcutaneous injection of drug four hours prior to an intraperitoneal inoculation of sporozoites and survival is monitored for a 30 day period. A similar procedure is followed for the oral administration of selected active compounds. Mice alive after 30 days are considered cured.

METHODS

ANIMALS

Male or female outbred ICR/HA Swiss mice (Mus musculus), six to seven weeks old, weighing 16 to 17 grams are used as test animals. They are maintained in groups of five and fed water ad lib.

Mice used as a source of gametocytes (donor mice) are eight weeks old and weigh 25 to 30 grams.

MOSQUITO COLONY

Anopheles stephensi are reared in an insectary maintained at 80°F and 70%

relative humidity with 14 hours of light and 10 hours of darkness. Larvae are fed a solution of 2.5% liver powder once a day. Emerged adults are fed a 10% glucose solution ad lib.

INFECTED MICE AS A SOURCE OF GAMETOCYTES

Donor mice to be used as a source of gametocytes are injected intraperitoneally with a dilution of infected heart blood from mice previously infected with sporozoites of Plasmodium yoelii yoelii 17X. These mice are used 2 to 3 days after inoculated with asexual blood parasites.

INFECTION OF MOSQUITOES

Mosquitoes are placed in a room maintained at 70°F and 70% relative humidity prior to the infected blood meal. Donor mice harboring a 2 to 10% parasitemia are anesthetized with Nembutal and placed on top of the mosquito cages for one hour to allow the mosquitoes to feed on infected blood. A second infected blood meal is given the following day and thereafter the mosquitoes are maintained on a 10% glucose solution. A single normal blood meal is given seven days after the first infected blood meal.

ISOLATION OF SPOROZOITES

Eighteen days after the first infected blood meal the mosquitoes are ether anesthetized, vacuumed into plastic bags and kept immobilized upon a cold table (-5° to 0°C). At this time the females are separated from the males and placed into a cold glass mortar. Males are discarded. After approximately 500 females are collected, one ml each of saline and mouse plasma are added and the suspension is macerated with a glass pestle for five minutes. An additional 20 ml of saline and mouse plasma (1:1) are added to the suspension and filtered through nylon monofilament screening fabric with a mesh opening of 90 microns. This removes large tissue fragments of the mosquitoes, yet allows the sporozoites to pass through into the refined suspension. This filtered sporozoite suspension is further diluted to contain approximately 250,000 sporozoites per 0.2 ml of inoculum.

ADMINISTRATION OF TEST COMPOUNDS

Each compound is ground with a mortar and pestle and then suspended in 0.5% hydroxyethylcellulose-0.1% Tween-80 to make the desired drug doses. The percent free base of each compound is not determined. Four hours prior to the inoculation of sporozoites, compounds are administered either subcutaneously or orally at three graded doses diluted four-fold (160, 40 and 10 mg/kg). Groups of five mice per dose level are used. Subsequent tests employing successive lower four-fold dilution are made if mice are cured at 10 mg/kg until the lower limit of a compound's activity is reached.

Infected control mice (receiving sporozoites only) begin to die from malaria starting seven days after inoculation of sporozoites. Deaths that occur prior to 7 days in mice treated with test compounds are considered drug toxicity deaths. A drug that is toxic for the host at each of the three initial dose levels is retested at doses diluted four-fold from 10 mg/kg.

INOCULATION OF MICE WITH SPOROZOITES

Mice are injected intraperitoneally with approximately 250,000 sporozoites. Twenty of these mice are divided into two groups of ten each. One group receives no drug and serves as a negative control. The other group is treated with WR 181,023 (125 mg/kg) and acts as a positive control. One additional group of five infected mice serving as a treated control is treated with chloroquine (100 mg/kg).

DETERMINATION OF ANTIMALARIAL ACTIVITY

After the mice have been inoculated with sporozoites they are placed in a room maintained at 84°F and 66% relative humidity. Antimalarial activity is determined by monitoring mortality daily. Mice alive after 30 days are considered cured.

RESULTS

CONTROLS

Mice inoculated with sporozoites but receiving no drug (negative control group) all routinely die within 7 to 12 days, as do mice receiving chloroquine. Mice serving as positive controls usually survive for the duration of the experiment (30 days).

COMPOUNDS TESTED AND DRUG ACTIVITY

For the period October 1, 1981 to April 15, 1982 756 three-level tests were performed. 39 compounds were active both orally and subcutaneously. 14 compounds were active only subcutaneously while six were active only orally. 25 compounds tested only subcutaneously were active. Two compounds were active when tested only orally. 577 compounds were inactive when tested only subcutaneously. 11 compounds were found to be inactive both subcutaneously and orally while two compounds were inactive when tested only orally.

In the period from October 1, 1980 through September 30, 1981 there were 1,656 three-level tests performed. 39 compounds were active both orally and subcutaneously. 17 compounds were active only subcutaneously while one was active only orally. 45 compounds tested only subcutaneously were active. One compound tested only orally was active. 1,297 compounds were inactive when tested only subcutaneously, while 33 compounds were found to be inactive both subcutaneously and orally. One compound was active when tested only orally.

SPECIAL TEST

A special test was performed to assess both the causal prophylactic (sporozoite challenge) and suppressive prophylactic (blood challenge) activity of five analogues of Floxacrine.

WR #	Bottle #
235,326	BH 96362
237,221	BJ 01091
237,942	BH 89652
226,626	BH 96344
243,251	BJ 45753

160 mg/kg of each analog was administered subcutaneously to 20 mice on Day 0 (D+0), a total of 100 mice plus 20 vehicle controls. On D+4 and D+7 the same five compounds were administered similarily to new groups of 20 mice each. On D+7 (2 hours after administration of the last drug one half of each group of 20 mice from D+0, D+4 and D+7 was inoculated intraperitoneally (I.P.) with $2.5X10^5$ sporozoites of Plasmodium yoelii(17X) while the other half were inoculated I.P. with $4X10^4$ parasitized erythrocytes of P. yoelii. Mortality was monitored for a 30 day period (until D+37).

The results of the mice surviving for the 30 days post parasite challenge are tabulated in Table VIII. The mortality data is tabulated in Table IX.

WR 237,221 and WR 243,251 retained full activity for a duration of 7 days. The other three compounds exhibited varying degrees of activity for 7 days.

TABLE VIII

Number of mice surviving to D+37 in groups receiving drug on either D+0, D+4 or D+7 after parasites challenge on D+7.

WR # Bottle #	D+7 Blood Challenge	D+7 Sporozoite Challenge	D+4 Blood Challenge	D+4 Sporozoite Challenge	D+0 Blood Challenge	D+0 Sporozoite Challenge
235,326 BH 96362	10	10	10	10	8	10
237,221 BJ 01091	10	10	10	10	10	10
237,942 BH 89652	10	10	7	9	3	9
226,626 BH 96344	5	10	4	8	3	7
243,251 BJ 45753	10	10	10	10	10	10
Vehicle controls	0	0	0	0	0	0

TABLE IX

Mortality data of mice receiving drug on either D+0, D+4 or D+7 after parasite challenge on D+7.

	Number of mice dead/day/died	
Day 7	Blood challenge	Sporozoite challenge
- Control	4/6 4/7 1/10 1/14	2/7 4/8 2/15 1/17 1/19
WR 226,626	2/18 1/19 1/23 1/28	
Day + 4		
- Control	3/6 3/7 3/9 1/14	7/9 1/10 2/15
WR 237,942	1/10 1/11 1/12	1/7
WR 226,626	3/9 1/15 1/20 1/24	1/10 1/15
Day 0		
- Control	1/6 7/7 1/9 1/11	5/9 1/11 1/14 2/18 1/19
WR 235,326	1/16 1/21	
WR 237,942	1/11 1/15 3/17 2/19	1/17
WR 226,626	2/7 2/9 2/15 1/17	1/10 1/17 1/21

A PROPHYLACTIC ANTIMALARIAL TEST SYSTEM INVOLVING THE TRANSFER OF PLASMODIUM YOELII YOELII EXOERYTHROCYTIC SCHIZONTS FROM RATS INTO MICE

To use of an effective and reliable screening procedure is necessary for the development of antimalarial drugs that are active against malarial exoerythrocytic schizonts. The exoerythrocytic schizont of malaria is the first multiplying stage of the parasite within the mammalian host in sporozoite induced infections. The exoerythrocytic schizont develops in relatively small numbers deep within the liver tissue of the host starting within seven hours and reaching maturity within 48 hours. To exoerythrocytic schizont has only one growth cycle in the host liver, so persistent blood schizonticidal drugs acting only on merozoites released from exoerythrocytic schizonts or subsequent asexual parasites within erythrocytes might give a false positive result. Therefore, a reproducible transfer method of infected liver cells after administration of drugs will tell the fate of the exoerythrocytic schizontal stage.

Foley and Vanderberg (Exp. Parasitol. 43, 1977) have described such a method for transmission of exoerythrocytic schizonts from rats into rats, mice and hamsters via intraperitoneal inoculation of minced liver.

Our method of rodent infection and subsequent transfer and Foley and Vanderberg's method differ in only one aspect. We used mashed female mosquitoes to liberate sporozoites for collection while Foley and Vanderberg dissected out the mosquito's salivary gland to collect sporozoites.

All hepatic exoerythrocytic donors were Sprague-Dawley rats weighing between 35 and 55 grams. All rats were raised in our laboratory's breeding colony of approximately 100 breeders. Donor rats were injected intravenously with $2\text{-}10X10^{5\text{-}6}$ sporozoites of P. yoelii yoelii 17X.

At the time when liver exoerythrocytic schizont inoculations were done (30-33 hours), the donors were sacrified by cervical dislocation and their livers were removed and sliced into fragments of 0.5 x 1.0 mm in cold tissue culture Medium L-15.

An amount equivalent to one-fifth of the donor liver was then inoculated I.P. into recipient rodents. Recipient rodents were either Sprague-Dawley rats (35-45 grams) or Swiss outbred mice (6-9 weeks old). The blood of all liver recipients and all remaining rats was examined once a week for two weeks.

# of Exps.	# of Sporozoites (Inoculated I.V.)	Liver Donor (% Infected)	Time of Exoerythrocytic Schizont Inoculation (hour)	Recipient Rodent	Liver Recipient % Infected
4*	$2.5\text{-}5.0 \times 10^6$	Rat (100%)	35-39	Mouse	33%
27*	$2.5\text{-}10 \times 10^6$	Rat (100%)	35-39	Mouse	25-35%
12	$2.5\text{-}10 \times 10^6$	Rat (100%)	31-33	Mouse	0-40%

* Experiments performed in previous year.

A SCREENING PROCEDURE FOR THE EVALUATION OF TRYPANOSOMICIDAL ACTIVITY OF CANDIDATE COMPOUNDS IN TRYPANOSOMA RHODESIENSE INFECTED MICE

According to the World Health Organization, there is no adequate information on the prevalence of human African trypanosomiasis. The best estimates report that 35 million people are exposed to the risk, and about 9,000 new cases are reported annually. African trypanosomiasis has a very high mortality rate and has considerable importance as a public health problem, especially in this age of increasing foreign travel.

No new antitrypanosomicidal drugs have been introduced during the past 24 years. Four drugs are currently available in the treatment of human trypanosomiasis caused by Trypanosoma rhodesiense or Trypanosoma gambiense. Three of these drugs, suramin, nitrofurazone and pentamidine are used in the treatment of the blood parasite, but lack efficacy in the treatment of central nervous system infections. The fourth drug, melarsoprol, is used in the treatment of central nervous system infections.

While these drugs are effective, they all have disadvantages. Suramin may cause renal damage, exfoliative dermatitis and has been shown to be teratogenic in rats. Nitrofurazone is toxic to the central nervous system and causes hemolytic anemia in glucose-6-phosphate deficient patients. Pentamidine may cause fatal hypertension, hypoglycemia and diabetes. Administration of melarsoprol leads to lethal encephalopathy in 10 to 15 percent of cases.

Occurring along with low therapeutic indices is the problem of trypanosomal drug resistance. Human trypanosome strains are commonly resistant to at least one chemotherapeutic agent and with some patients their infection is resistant to all known drugs.

Therefore, there are definite needs to develop and test compounds that are potentially active against resistant strains and that are less toxic than the existing drugs. Further testing also needs to be done using different routes of administation and combinations of more than one drug.

The test system described herein was developed specifically to evaluate the trypanosomicidal activity of large numbers of candidate compounds. Based on blood induced T. rhodesiense infections in mice, it acts as a primary screen or as a secondary screen and confirmatory test and gives precise quantitative evaluations of chemical compounds that demonstrate potentially useful therapeutic and/or prophylactic activity in T. rhodesiense infections. Consequently, it is also a helpful guideline in the synthesis of new active agents.

All candidate compounds were obtained from the Department of Parasitology at the Walter Reed Institute of Research and included:

1. Chemicals structurally related to compounds of known value in the treatment or prevention of T. rhodesiense infections;

2. Chemicals structurally unrelated to compounds of known value in the treatment or prevention of T. rhodesiense infections;

3. Structural analogues of compounds that have demonstrated activity in our screening procedure and represent novel chemical groups;

4. Compounds known to have activity against other infectious agents.

METHODS

ANIMAL HOSTS

ICH/HA Swiss mice (Mus musculus) used in this screening procedure weigh 28 to 30 grams with weight variations in any given experimental or control group carefully limited to three grams. In all tests the animals are of the male sex and approximately the same age.

Animals are housed in metal-topped plastic cages, fed a standard laboratory diet and given water ad lib. After drug treatment, mice are kept in a room maintained at a temperature of 84° (±2° F) with a relative humidity of 66% (±2%).

TEST PROCEDURE

Test animals receive an intraperitoneal injection of 0.2 cc of a 1:15,000 dilution of heparinized heart blood drawn from a donor mouse infected three days earlier (approximately 13,000 - 17,000 trypomastigotes).

The donor line is maintained by three-day blood passes; each animal receives 0.1 cc of a 1:500 dilution of heparinized heart blood drawn from a three-day donor.

One group of infected, untreated mice is included as a negative control to check factors such as changes in the infectivity of our T. rhodesiense strain or in the susceptibility of the host. In order to determine the effect a drug exerts on a trypanosome infection, two parameters are measured; the increase in mouse survival time and drug curative action. For comparative purposes, two standard compounds, stilbamidine isethionate and 2-hydroxystilbamidine isethionate, are administered subcutaneously at one level each (26.5 mg/kg) to separate groups of ten mice. The same positive controls are administered at 53 mg/kg orally when new compounds are tested orally. These diamidines serve as positive controls, producing definite increase in survival time and curative effects. Another function of the two positive controls involves a check on whether three procedures are performed correctly; the drug weighing, the preparation of drug solutions and suspensions, and the administration of drugs.

DRUG ADMINISTRATION

Test compounds are dissolved or suspended in peanut oil before they are administered subcutaneously. Compounds to be administered orally are mixed in an aqueous solution of 0.5% hydroxyethylcellulose-0.1% Tween-80.

Treatment consists of a single dose given subcutaneously or orally two to three hours after the injection of parasites. Deaths that occur before the fourth day, when untreated controls begin to die, are regarded as a result of action by the drug, not parasites.

Each compound is initially administered in three graded doses diluted four-fold

to groups of five mice per dose level. The top dose is 424, 212, or 106 mg/kg, depending on the amount of compound available for testing. Active compounds are subsequently tested at six or nine dose levels, diluted two-fold from the highest dose. Successive six-level tests are performed at respectively lower doses if necessary until the lower limit of activity is reached.

A drug that is toxic for the host at each of the three levels initially tested is retested at six dose levels diluted two-fold from the lowest toxic dose.

DRUG ACTIVITY

Acceptance of a drug as being sufficiently active for detailed studies is predicated on the margin between the maximum tolerated dose (MTD) and the minimum effective dose (MED) producing a significant effect. A MTD is defined as the highest dose up to 424 mg/kg causing no more than one of five animals to die from drug toxicity. The MED is defined as the minimum dose increasing the life span of treated animals by 100% over the life span of untreated controls.

Clearly inactive compounds are rejected after one test; border-line compounds after two tests. Active compounds are characterized by dose-response curves, which establishes the spread between the MTD and the lower limit of activity by a determination of drug activity in the dose level dilution tests. Treated animals alive at the end of 30 days are considered cured.

RESULTS

Table X summarizes the number of compounds tested and the number of mice used from August 1, 1972 through April 7, 1982.

The compounds tested during the period of October 1, 1981 through April 7, 1982 numbered 935. Seventeen compounds were identified as having chemotherapeutic activity. Included in these compounds, 16 were only active subcutaneously and one was active orally. The one compound that was reported as active orally, T-21809, was active subcutaneously in the previous reporting period. This compound has not been reported as active both orally and subcutaneously in either report.

The compounds tested during the period of October 1, 1980 through September 30, 1981, numbered 1,222. Sixty-two compounds were identified as having chemotherapeutic activity. Included in these compounds, 50 were only active subcutaneously, 6 were only active orally and 6 were active both orally and subcutaneously. Two compounds (T-18151 and T-19781) that were reported as only active orally in this reporting period were active subcutaneously the previous reporting period. These two compounds have not been reported as active both orally and subcutaneously in either report.

The compounds tested during the period of October 1, 1979, through September 30, 1980, numbered 3,462. Eighty-eight compounds were identified as having chemotherapeutic activity. Included in these compounds, 78 were active subcutaneously, 3 were only active orally and 7 were active both orally and subcutaneously.

The compounds tested during the period of October 1, 1978, through September 30, 1979, numbered 2,783. One-hundred and twenty-five compounds were identified as having chemotherapeutic activity. Included in these compounds, 116 were only active subcutaneously, 7 were only active orally, and 2 were active both orally and subcutaneously. One compound that was active orally this reporting period was active subcutaneously the previous reporting period. This compound has not been reported as active both orally and subcutaneously in either report.

The compounds tested during the period from October 1, 1977 through September 30, 1978, numbered 3,032. Ninety-one were recognized as active compounds: 14 of these were active both orally and subcutaneously; 9 were active only orally, 54 were active only subcutaneously.

4,235 compounds were tested from June 1, 1976 to September 30, 1977. 396 compounds were recognized active: 109 were active both orally and subcutaneously; 17 were active only orally; 270 were active only subcutaneously; 17 were active only subcutaneously.

1,653 compounds were tested during the period of June 1, 1975 through May 31, 1976. 257 compounds were recognized as active; 198 were active subcutaneously and 59 were active orally.

1,826 compounds were tested in the period of June 1, 1974 through May 31, 1975. 298 compounds were recognized as active; 225 of these were active subcutaneously and 73 were active orally.

1,581 compounds were tested in the period during June 1, 1973 through May 31, 1974. 185 of these compounds were recognized as active; 92 were active subcutaneously and 93 were active orally.

3,030 compounds were tested in the period of June 1, 1972 through May 1, 1973. Sixty-eight of these compounds were recognized as active.

The activity evaluations provided by our screening procedure are precise and quantitative, therefore the above breakdowns are significant when considering the following:

a) The dose-response curves of active compounds administered subcutaneously reveal a wider spread between the MTD and the MED than those of active compounds administered orally.

b) These dose responses also display a wider spread of toxic effects when active compounds toxic for the host are administered subcutaneously rather than orally.

TABLE X

Trypanosoma rhodesiense

Compounds Tested and Mice Utilized

August 1, 1972 - April 7, 1982

Date	Number of Three-Level Tests	Number of Mice
October 1, 1981 - April 7, 1982	935	14,785
October 1, 1980 - September 30, 1981	2,043	32,520
October 1, 1979 - September 30, 1980	4,780	60,110
October 1, 1978 - September 30, 1979	3,158	49,708
October 1, 1977 - September 30, 1978	4,025	64,600
June 1, 1976 - September 30, 1977	4,235	73,280
June 1, 1975 - May 31, 1976	1,653	30,290
June 1, 1974 - May 31, 1975	1,826	33,850
June 1, 1973 - May 31, 1974	1,581	25,360
August 1, 1972 - May 31, 1973	3,030	51,405
TOTAL	27,266	435,908

DRUG-RESISTANT TRYPANOSOME LINES

The resistance of T. rhodesiense to selected antitrypanosomal compounds can be induced by repeated drug pressure in an in vivo test system. This is achieved by infecting mice with a standard inoculum of parasites, administering the test compound in a dose just below the curative level, and passing parasites from these animals to a new set of mice when the parasitemia rises to a desirable level. Passes are made every three to four days with drug doses being increased as resistance develops at each dose level.

This type of study can establish the rate at which T. rhodesiense acquires resistance in mice to selected compounds. Cross resistance to trypanosomicidal compounds found to be active against the drug-sensitive line in primary screening tests may also be determined.

Lines of trypanosomes have been developed which are completely or partially resistant to the following compounds.

Completely Resistant	Pass #	Highest Dose Achieved
Pentamidine	212	212.0 mg/kg
Melarsoprol	128	424.0 mg/kg
Suramin	129	543.0 mg/kg

METHODS

ANIMALS

Male of female ICR/HA Swiss mice (Mus musculus) of approximately the same age and weight are used in all procedures. Animals are housed in groups of five, fed a standard laboratory diet and given water ad lib. Mice are kept in a room maintained

at 84°F (±2°F) and a relative humidity of 66% (±2%).

DEVELOPMENT AND MAINTENANCE OF DRUG-RESISTANT LINES

On day zero. fifteen male or female mice are divided into three groups of five animals. All animals are initially inoculated intraperitoneally with drug-sensitive T. rhodesiense (Wellcome CT-strain) trypomastigotes in saline diluted blood (1:500) drawn from a previously infected donor mouse. Group I serves as a negative control, receiving no drug. Group II receives drug either orally or subcutaneously on day 0 and day 1. Group III is given the same dose of drug by the same route on day 0 only. On day 3 or 4, 15 new mice are infected with saline-diluted blood (1:500) from Group II. The pass is made from Group III if Group II animals demonstrate no parasites upon blood examination. These newly infected mice are similarly divided into three groups and given the same drug regimen as that just described. Passes are thus made every three of four days from the most recently infected and treated groups of animals. Drug doses are increased as resistance develops.

Once complete resistance to the highest tolerated dose of the compound is reached, the line is passed two times each week using two groups of five mice. Group I mice receive no drug and serve as a negative control. Group II mice receive a low dose of drug to maintain drug pressure and serve as donor mice for the next pass.

TEST PROCEDURE - INOCULATION OF PARASITES

Giemsa-stained blood smears from donor mice infected three days earlier with T. rhodesiense trypomastigotes are microscopically examined to determine parasitemias (i.e., number of trypomastigotes in a field of 100 erythrocytes). One set of test animals is infected with the drug-sensitive line of parasites and receives an intraperitoneal injection of 0.2 cc of a 1:15,000 dilution of heparinized heart blood drawn from a donor mouse harboring a parasitemia of 30-35% (approx. 13,000-17,000 trypomastigotes). Other sets of mice are similarly infected with each drug-resistant line to be tested. Blood dilutions are made such that all mice infected with the resistant lines receive approximately the same number of trypomastigotes as mice infected with the drug-sensitive line.

One group of ten infected mice from the sensitive line and from each resistant line serve as negative control, receiving no drug.

TEST PROCEDURE - COMPOUND ADMINISTRATION

Test compounds are mixed in either peanut oil for subcutaneous administration or 0.5% hydroxyethylcellulose-0.1% Tween-80 for oral administration. Compounds are given immediately following challenge with trypomastigotes.

Compound doses are diluted two or four-fold from a level that has been projected to be fully curative (should sufficient quantities be available). Five mice are used for each dose level.

COMPOUND ACTIVITY

Mortality is used as an index of drug activity. Untreated negative control mice routinely die on days 4 or 5 after inoculation of parasites. Increases in life span relative to that of negative controls at each dose level are recorded. Curative activity is used in assessing the level of resistance of selected compounds. Mice surviving for 30 days are considered cured.

RESULTS

There were nine experiments (#'s 33-41) performed during this period. Composite results are summarized below and the degree of resistance are presented in Table XI.

Thirty compounds that had been previously tested and showed activity in the T. rhodesiense primary test system, were tested for activity against either one or more of three drug-resistant T. rhodesiense lines. The drug-resistant lines of T. rhodesiense consist of trypanosomes resistant to either pentamidine (>212 mg/kg), melarsoprol (>424 mg/kg) or suramin (>543 mg/kg).

The pentamidine-resistant T. rhodesiense line was cross resistant to all drugs tested against it except for the antimonial (T-11510), the phosphonium (T-10583) and

the naphthalene, suramin (T-21824). The melarsoprol-B resistant T. rhodesiense line was cross resistant to all drugs tested against it except suramin. The suramin resistant T. rhodesiense line was sensitive to all of the drugs tested against it except suramin.

THE LOSS OF DRUG RESISTANCE OF 2 LINES OF T. RHODESIENSE ASSOCIATED WITH REMOVAL FROM DRUG PRESSURE.

T. rhodesiense has the ability to develop resistance to a number of commonly used anti-trypanosomal chemotherapeutic agents. As stated previously in this report, one line of trypanosomes is resistant to suramin, another resistant to melarsoprol and a third is resistant to pentamidine.

This report is interested in the time it takes, for two of these resistant lines (suramin and melarsoprol) to lose resistance to their respective drugs. The two trypanosoma lines that were removed from drug pressure will be tested at intervals of 1, 2, 3, 6, 9, 12 etc. months for loss of resistance by comparison with the original drug-resistant line maintained under drug pressure and the drug-sensitive RR-line.

The test procedure, administration of drug, and determination of drug activity is the same as the previous section on Drug-Resistant Trypanosome Lines.

At this time of writing, the first one month interval has not been reached, therefore no results have been obtained.

TABLE XI

Summary of degrees of resistance[a] of drug-resistant lines of Trypanosoma rhodesiense to selected antitrypanosomal compounds tested between October 1, 1981 to March 31, 1982.

Class of Compound	Mum No.	Bottle No.	Degree of Resistance[a] Pentamidine-Resistant Line	Mel-B-Resistant Line	Suramin-Resistant Line
Acridine	T-12703	AB-76740	>4.0	>4.0	1.0
Antimonial	T-11510	AS-21026	1.0	-	-
Bisquinoline	T-11396	BJ-46054	>255.4	>255.4	1.0
	T-18007	BJ-39273	>1009.3	>1009.3	1.0
	T-18008	BJ-39282	>1009.3	>1009.3	4.0
	T-18130	BJ-42510	>255.4	>255.4	4.0
	T-18131	BJ-44783	>1009.3	>1009.3	1.0
	T-18132	BJ-44792	>255.4	>255.4	1.0
	T-18923	BJ-45833	>255.4	>255.4	1.0
	T-18929	BJ-50861	>255.4	>255.4	1.0
Diamidine(Stilbamidine)	T-10977	BH-96657	>1034.1	>258.5	4.0
	T-12672	BH-39750	>127.3	-	-
(Pentamidine)	T-13344	BG-11391	>424.0	>255.0	4.0
	T-16117	BJ-08661	>64.0	-	-
(Berenil)	T-16245	BJ-92341	-	>64.4	1.0
	T-17156	BJ-33664	>530.0	-	-
	T-20755	BJ-63279	>106.0	>963.6	1.0

TABLE XI (Cont.)

Class of Compound	Mum No.	Bottle No.	Degree of Resistance[a] Pentamidine-Resistant Line	Mel-B-Resistant Line	Suramin-Resistant Line
Diamidine Intermediate	T-19781	BJ-59006	>106	>258.7	4.0
Furan(Lampit)	T-15937	BJ-07691	-	-	1.0
Naphthalene(Suramin)	T-21824	BK-01916	1.0	1.0	255.4
Phenanthridine(Eth-Br)	T-12709	BE-19397	>252.4	-	-
Phosphonium	T-10583	AS-67317	2	-	-
	T-10595	AT-33487	ND[b]	-	-
Purine	T-10656	BG-80510	>4	>4	-
Quinazoline	T-15705	BJ-42672	>8.0	-	-
	T-15707	BJ-01939	>16.0	-	-
Terephthalanilide	Mum-3679	BH-62987	>32.0	16.0	1.0
Triazine(Mel-B)	T-10656	BJ-90909	>255.0	>3854.6	3.7
Arsenicals	T-17850	BJ-39764	>8.0	>16.0	-
(Mel-Ni)	T-21778	BJ-91531	>106.0	>3854.6	3.7

[a] Degree of resistance is determined by comparing CD_{50} values for each compound when tested against the sensitive and drug-resistant line (degree of resistance = CD_{50} of resistant line $\div$ CD_{50} of sensitive line).

[b] ND = Degree of resistance is not determinable from current data.

THE CHEMOTHERAPY OF EXPERIMENTAL CHAGAS' DISEASE

Infections by the haemoflagellate Trypanosoma cruzi, the etiologic agent of Chagas' disease, present a devastating public health problem for millions of people in Central and South America. Reduviid bugs transmit T. cruzi to man via the insect's infected feces causing a disease characterized by an acute phase and a subsequent chronic degenerative phase. In addition to the cultural, social and economic factors that make Chagas' disease particularly difficult to manage, the problem is compounded by this protozoan's cellular invasiveness and its pleomorphic morphological and biochemical nature. No satisfactory course of drug therapy has been found that will treat all stages of the infection while remaining non-toxic to humans.

The unavailability of effective chemotherapeutic agents for the control of T. cruzi infections in man prompted us three years ago to develop a mouse model for screening potential antitrypanosomal drugs. Preliminary studies with three strains of T. cruzi and two genetically different types of mice led to the development of a reliable screening test employing C_3H mice infected with EP-strain of T. cruzi. Several new classes of compounds were found to be active against T. cruzi infections in mice. Mouse survival time and mortality during the acute phase of infection was used as an index of drug-schizotrypanocidal activity.

In September of 1978 the test system was revised to its present status by using Swiss mice as hosts and the Y-strain of T. cruzi (obtained from Dr. Dvorak at N.I.H.) as the infective agent. Thus, the current screening program conveniently employs randomly bred Swiss mice (ICR/HA strain) from our own breeding colony and an established strain of parasite that is well suited to the requirements of this drug-screening program.

The test system described herein serves to evaluate prospective chemotherapeutic agents against experimental Chagas' disease by obtaining the following information:

1) Compound suppressive activity. The degree of suppressive activity is given by the mean percent increase in survival time compared with infected untreated controls.

2) Compound curative activity. Mice surviving 40 days after infection and treatment are considered cured when confirmed by secondary experimental procedures currently under consideration.

3) Compound toxicity. Death of treated mice before negative controls begin to die is attributed to compound toxicity.

4) An estimate of the compound's therapeutic index.

TEST PROCEDURE

On day zero, male ICR/HA Swiss randomly bred mice six to seven weeks old, weighing 18-22 grams, are inoculated intraperitoneally with approximately 1.3×10^5 trypomastigotes in blood drawn from donor mice infected one week previously with T. cruzi trypomastigotes (Y-strain). The mice are raised in this laboratory. Within 30 minutes following challenge, mice are given a single subcutaneous injection of the test compound mixed in peanut oil. Each compound is initially tested at three dose levels, usually 640, 160 and 40 mg/kg unless known toxicity data suggest lowering the top dose. The end point in activity of each active compound will be determined. Mortality is recorded daily for a period of 40 days after the challenge with parasites. Blood smears will be taken on day 40 to see if any trypomastigotes are present.

Infected negative controls receive an injection of the vehicle alone. This group consistently dies within 9-15 days after the intraperitoneal inoculation of parasites. A positive control drug is included in each experiment. The nitrofuran, Lampit, known to have limited therapeutic value in treating patients with Chagas' disease, is currently being used as a positive control. It has been found to delay the onset of mortality as well as allowing some mice to survive the 40 day period after being administered once subcutaneously at a dose of 640 mg/kg.

A classification system is used to assess the relative activity of prospective compounds by comparing the life span of treated animals to the longevity of negative controls. Schizotrypanocidal activity is divided into three categories; positive, minimal and negative. A positive compound is one producing at least a 50% increase

in life span of mice over that of controls. A minimal compound produces a 20 to 49.9% increase in longevity, and a compound producing less than a 20% increase in life span is considered negative.

Active compounds prevent or delay acute mortality. The test system as designed does not assure that mice living past the 40 day observation period are cured; if complete elimination of the parasite is not attained during the acute stage of infection and the animal survives, a chronic stage follows.

RESULTS

CONTROL GROUPS

Mice infected but receiving no test compound (negative control group) routinely die between days 9 and 15 after injection of trypomastigotes.

Mice inoculated with trypomastigotes then treated with the nitrofuran, Lampit, at 640 mg/kg serve as a positive control group. Some of these mice usually survive for the duration of the experiment, 40 days, and those who don't have a prolonged survival time.

EXPERIMENTAL COMPOUNDS

In experiments 98 through 126 covered in this report there were 138 three-level tests performed.

There were eight active compounds, some of which tested in three-level tests and some in six-level tests.

BG-55008	ZP-25978
BJ-90810	AK-37958
BG-91040	BH-13989
BJ-75484	BK-15367

There were 94 negative three-level tests performed and 15 negative six-level tests performed.

A C K N O W L E D G M E N T

The personnel at the Rane Laboratory participating in this Chemotherapy of Malaria project deserve a tremendous degree of credit for an excellent performance.

CHEMOTHERAPY ASPECTS

Joaquin Ardavin
Esther Caballero
Delia Febles
Rosa Fontela
Concepcion Gutierrez
Hortensia Salvador
Merida Ventura
Catalina Zaldivar

CARE AND MAINTENANCE OF ANIMAL COLONY

Maria Chavez
Maria Dominguez
Paul Lee
Nancy Oliva
James Phillips
Phillip Roberts
Frank Wilson

MAINTENANCE OF LABORATORY COMPLEX

Francisco Fabricio

ADMINISTRATIVE COORDINATOR ANNUAL REPORT TYPIST

Maria Isabel Antonini

ASSISTANT DIRECTOR

Richard G. May

Arba L. Ager, Jr.
Principal Investigator

DISTRIBUTION LIST

12 copies	Director Walter Reed Army Institute of Research Walter Reed Army Medical Center ATTN: SGRD-UWZ-C Washington, D.C. 20012
4 copies	Commander US Army Medical Research and Development Command ATTN: SGRD-RMS Fort Detrick, Frederick, MD 21701
12 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDA Cameron Station Alexandria, VA 22314
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20014
1 copy	Commandant Academy of Health Sciences, US Army ATTN: AHS-CDM Fort Sam Houston, TX 78234

END

FILMED

2-85

DTIC